April 1, 2004

Ms. Karlene Fine
Executive Director
North Dakota Industrial Commission
State Capitol
600 East Boulevard Avenue, Department 405
Bismarck, ND 58505-0840

Dear Ms. Fine:

Subject: EERC Proprosal No. 2004-0041; "The Health Implications of the Mercury–Selenium Interaction"

Enclosed are the original and six copies of the subject proposal. The PDF of the file was e-mailed to you. Also enclosed is the $100 application fee.

If you have any questions or comments, please contact me by phone at (701) 777-5066 or by e-mail at nralston@undeerc.org.

Sincerely,

Nicholas V.C. Ralston
Research Scientist

NCR/bak

Enclosures

c/enc: Harvey Ness, Lignite Research Council

THE HEALTH IMPLICATIONS OF THE MERCURY–SELENIUM INTERACTION

EERC Proposal No. 2004-0041

Submitted to:

Ms. Karlene Fine

North Dakota Industrial Commission
State Capitol
600 East Boulevard Avenue, Department 405
Bismarck, ND 58505-0840

Amount of Request: $50,000

Submitted by:

Nicholas V.C. Ralston
Laura J. Raymond
Steven A. Benson

Energy & Environmental Research Center
University of North Dakota
PO Box 9018
Grand Forks, ND 58202-9018

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Nicholas V.C. Ralston, Research Scientist

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Dr. William D. Gosnold Jr., Interim Director
Office of Research and Program Development

April 2004

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ABSTRACT

The research community acknowledges the robust nutritional selenium status of U.S. citizens is an important asset in maintaining public health since dietary selenium enhances cancer resistance and supports the immune system. It is also known that selenium is beneficial in protecting against mercury exposure since research in animal models has established that selenium deficiency increases vulnerability to mercury toxicity while enhanced dietary selenium status is protective. However, the biochemical mechanism for selenium's protective effect against mercury remains unclear. Selenium clearly influences mercury metabolism, but mercury's impact on selenium physiology appears to be equally important. Recent studies have shown that mercury exposure diminishes the activity of selenium-dependent enzymes. It is reasonable to consider that the influences mercury and selenium have upon one another may share a common basis in the exceedingly high binding affinity between these elements.

This proposal describes a 2-year multiclient-funded research program that will be unique in exploring the interactions between mercury and selenium in experimental models designed to closely approximate human patterns of exposure. The proposed studies will examine the effects of dietary intakes of methylmercury and the protective effects of dietary selenium. This research project will resolve important questions regarding the significance of mercury–selenium interactions in human health.

This proposal describes a series of complementary investigations that will be performed to address these questions in a research program costing a total of $153,846 ($50,000 from the North Dakota Industrial Commission; $50,000 in funds from cost-share partners; and $53,846 from U.S. Department of Energy Jointly Sponsored Research Program) that will involve Dr. Nicholas V.C. Ralston, Dr. Laura J. Raymond, and Dr. Steven A. Benson of the Energy & Environmental Research Center.

TERMS AND ABBREVIATIONS

in silico – biochemistry studies carried out in a computer-based simulation or mathematical model, particularly studies relating to or emulating biological reactions at the molecular level.

in vitro – biochemistry studies carried out in an artificial environment such as a test tube rather than inside a living organism in order to more precisely define activities (from Latin, literally "in glass").

in vivo – biochemistry studies carried out inside a living organism, as in an experimental study of animal biochemistry or behavior (from Latin, literally "in the living").

lysosome – a compartment present within all cells that is responsible for chemical breakdown for recycling of building blocks of cellular components such as lipids and proteins. Materials that resist chemical breakdown tend to accumulate in lysosomes.

mercury – a naturally occurring element that exists in three forms: elemental (uncharged, volatile), oxidized (+2 charged, capable of chemically binding with two partners), and organic (most commonly encountered in nature as chemically bound to a methyl group and another partner such as a selenium, sulfur, or carbon).

mercury selenide – mercury chemically bonded with a reduced form of selenium, forming a virtually insoluble (solubility coefficient 10^{-57}) mineral known among geologists as tiemannite.

methylmercury – mercury that is chemically bound to a carbon associated with three hydrogens, formed by bacteria and subsequently accumulated and magnified up the food chain in fish.

selenium-dependent enzyme – a selenoprotein with known specific chemical activities, also referred to as a selenoenzyme. Selenoenzymes perform functions in all cells of the body.

selenium-dependent protein – any protein with a specific requirement for selenocysteine; also referred to as a selenoprotein. 20–30 selenoproteins are expressed in the body; group includes selenoenzymes as well as proteins whose biological activities remain uncharacterized.

selenocysteine – the 21st amino acid**,** homologous to cysteine, with selenium substituted for the sulfur. While all other amino acids are repeatedly used in protein synthesis (cellular proteins are continually broken into their component amino acids and resynthesized, sometimes many times a day), selenocysteine must be completely broken down into free selenide and resynthesized for each cycle of selenoprotein synthesis.

selenomolecule – selenium-containing molecules that are far smaller than selenoproteins; these low molecular weight species are remarkable for their apparent high abundance in brain tissues. Their chemical composition, structure, and functions remain unknown at present, but ~20% of the selenium found in brain tissues appears to be in the form of these small molecular species.

THE HEALTH IMPLICATIONS OF THE MERCURY–SELENIUM INTERACTION

PROJECT SUMMARY

The primary objective of this project is to clarify the biochemical mechanism for dietary selenium's protective effect against mercury toxicity. This is important since selenium status can vary between regions of the globe and influence the sensitivity or robustness of a population to mercury exposure. Since the selenium status of American citizens is accepted to be quite good, their protection against mercury exposure is expected to be greater. This being the case, it is important to define the extent of the influence of selenium status on the threshold levels below which mercury exposure is harmless so that decision makers can be informed and properly determine regulatory and legislative policy.

Selenium and mercury are known to influence one another's retention and distribution in tissues. However, the essential experiments needed to properly characterize the nature of the interactions between dietary selenium and methylmercury have not been done. To help correct this omission, the in vivo investigation conducted in Study 1 of this project was designed to determine the mutual influences of mercury and selenium on one another's uptake, retention, distribution, and excretion. In Study 1, laboratory rats will be fed diets prepared with selenium at levels that are deficient, adequate, or enriched. Each diet will be supplemented with mercury at 0, moderate, or toxic concentrations in a 3 × 3 factorial study of selenium and mercury dose effects. Rats will be terminated after consuming the test diets, and reference and vulnerable test tissues will be collected and analyzed for mercury and selenium content as well as selenium-dependent enzyme activity.

Study 2 will use chromatographic methods to compare mercury binding to selenium versus sulfur and will extend this to investigation of mercury binding to biologically important

selenium-containing biomolecules. The results of this study will be used to form a more complete understanding of the effect of selenium in protection against consequences of mercury exposure on selenium-dependent metabolic processes than has been attainable using less direct methods.

Our computational modeling investigation (Study 3) will use the mercury and selenium values obtained from our animal and chromatography studies as a database for establishing a computational model of mercury–selenium interactions, enabling us to predict the quantities and effects of mercury accumulation in the tissues and their association with low molecular weight selenomolecules. This database will be used to extend our insights into the biochemical interactions between mercury and selenium observed in these animal models to projections of the extents of these same reactions in cases of historic human exposure used for establishing benchmark doses in an effort to evaluate the validity of current and future risk assessments. Additional sponsors have expressed confidence they will join in funding the expansion of Year 2 research. The increased scope of research studies that are anticipated to be supported by this additional funding is described in Appendix A.

PROJECT DESCRIPTION

Since the major issue of public concern regarding mercury exposure is the methylmercury present in fish consumed by humans, it is important to replicate this route of exposure using experimental models that employ dietary selenium and mercury present at meaningful concentrations for determination of their interactions and effects. Although a tremendous amount of work has been done with the intent of examining methylmercury exposure and the protective influence of selenium, few of the studies performed to date have attempted to replicate the

normal dietary exposure route of these elements, and no studies have closely examined the effects of these elements on the distributions of one another in the tissues.

Previous studies of the interactions between mercury and selenium have typically used injections of physiologically inappropriate molecular forms and concentrations of mercury and selenium instead of chronic dietary exposure as occurs in humans. No previous study has examined the effect of selenium status and methylmercury exposure on mercury–selenide complex formation as we propose to do. Nor has any previous study examined the distribution of these elements in the pituitary, a particularly important and highly vulnerable tissue (see Appendix A), let alone considered the effects of mercury in pituitary selenoenzyme metabolism. The proposed studies will apply highly sensitive methods that will reveal the magnitude of the effects of selenium status on mercury complex formation and maintenance of selenoenzyme activity. The in vivo examination of the physiological interactions of methylmercury and selenium conducted in Study 1 is specifically designed to allow us to rapidly build a database of meaningful information that will be applied in developing our computational model. In vitro examinations of binding between mercury- and selenium-containing species will be conducted in Study 2, providing a database for the molecular basis of our computational assessments. The in silico investigation performed in Study 3 will correlate the data acquired from our in vitro and in vivo studies.

Study 1: Accumulation and Distribution of Selenium and Methylmercury.

This study is designed to investigate the mutual influences of mercury and selenium on one another's uptake, retention, distribution, and excretion in vivo. Laboratory rats will be fed torula yeast-based diets prepared with selenium at levels that are deficient (0.1 mM), adequate (1.0 mM), or enriched (10.0 mM). Each diet will be supplemented with mercury added at

0.0, 5.0, or 50 mM (approximately 0, 1.0, or 10 ppm Hg, respectively) in a 3 × 3 factorial (nine dietary treatment groups) study of selenium and mercury dose effects. Fifty-four Long Evans rats will be randomly assigned to nine weight-matched groups (six rats per treatment group) that will be fed these diets for 8 weeks. On Day 56, the animals will be dissected, and the selenium and mercury concentrations will be assessed in the reference tissues: blood, liver, and kidney, as well as in tissues known to be vulnerable to mercury accumulation, the pituitary, testis, and brain. In these latter tissues, the molar distribution of mercury and selenium in the total tissue as well as the distributions in the low molecular weight fraction expected to accumulate residual Hg–Se complexes will be measured. Selenium concentrations in these samples will be determined using graphite furnace atomic absorption spectroscopy (GFAAS), while mercury concentrations will be determined using cold-vapor atomic absorption spectroscopy (CVAAS) by the Analytical Research Laboratory at the Energy & Environmental Research Center (EERC).

Study 2: Methylmercury Binding Affinities to Thio- and Selenomolecules.

Mercury's high affinity for sulfur is reflected in the term used to designate molecules that contain sulfur. These molecules are generically referred to as mercaptans, meaning literally "(substance) that captures mercury" through formation of the sulfur–mercury bond. Although mercury's binding affinity for sulfur is quite high, it is recognized that selenium forms complexes with much higher affinities. Unfortunately, previous work on this topic has focused on inorganic sulfur and selenium molecules and omitted many biologically meaningful molecular species. This study will examine and compare the binding affinities between comparable molecular forms of selenium and sulfur molecules with methylmercury under conditions that reflect the biological range of their cellular concentrations, using physiologically appropriate pH and ionic concentrations that reflect the intracellular environment.

High-performance liquid chromatography (HPLC) will be used to separate free and bound species of selenium- and mercury-containing molecules that will be analytically quantitated. Using this experimental model, the interactions between selenium and mercury versus sulfur and mercury will be quantitatively compared and evaluated to accurately and precisely determine their respective binding affinities.

Organic and inorganic forms of mercury (methylmercury vs. $HgCl_2$), as well as organic and inorganic forms of selenomolecules and comparable sulfur-containing molecules will be repeatedly tested in multiple parallel assessments designed to define the range of physiological conditions expected to influence these binding interactions. Means and standard deviations at each analysis point will be evaluated, and trends for independent effects will be calculated and plotted for regression analysis.

Study 3: Determination of Biochemical Threshold of Methylmercury Effects.

Our computational modeling study will use the data obtained from Studies 1 and 2 as a primary database for establishing a computational model of mercury–selenium interactions in order to quantify the distribution and molecular associations of mercury. This study will determine the mercury species that accumulate in the tissues, particularly those that form complexes with low molecular weight selenomolecules. This database will be used to extend our insights into the biochemical interactions between mercury and selenium observed in our in vitro and in vivo models to projections of the extents of these reactions in cases of historic human exposure. Since these cases have been used for establishing benchmark mercury doses, this effort to evaluate the influence of selenium status on these parameters will help establish the validity of current and future risk assessments.

This study will apply computational determinations of molar ratios of mercury and selenium determined through analysis of their relative tissue concentrations and distributions. A compartmental model of these tissue distributions will be used to define the availability of the major molecular participants: mercury and methylmercury in association with selenocysteine, selenide, selenophosphate, cysteine, sulfide, etc., and apply our calculated binding constants to assess the relative percent bound and free selenium in each tissue compartment. Data regarding tissue trace element distributions will be supplemented with thorough analysis of selenium and mercury in tissues compiled from our other data sources in a growing database that compares these relationships in a macroanalysis tool useful for projecting the effects of mercury in human populations that differ in selenium status.

STANDARDS OF SUCCESS

The criteria for defining success in this research program will be the extent to which these studies provide new information regarding the effect of mercury on selenium-dependent physiological processes and the effect of dietary selenium on mercury distribution and disposition in the body. Study 1 will be successful by defining the relative effects of selenium and methylmercury on one another's absorption and tissue distribution. Study 2 will be successful through analytically determining the binding affinities between mercury and selenoproteins. Study 3 will achieve its objectives by quantitatively comparing the extent of mercury-dependent diversion of biologically available selenium in tissues and establishing a database that will provide useful information in predicting the extent of selenium-dependent protection against mercury and its influence relative to benchmark mercury dose levels.

The EERC is committed to delivering consistent, high-quality research results through performance of this project that will advance the scientific understanding of the relationship

between mercury–selenium interactions and human health. Procedures and instrument calibrations in the Environmental Health Research and Analytical Research Laboratories follow nationally recognized or approved standards and methods. These laboratories have quality assurance and quality control protocols in place to ensure the assays applied in this project are properly implemented and high-quality data are obtained.

BACKGROUND

Mercury is a heavy metal that is of significant concern as a global pollutant. The toxic effects of methylmercury can make it a health problem, and it is listed by the International Program of Chemical Safety as one of the most dangerous chemicals in the environment (1). Based on the current understanding of mercury toxicity, the U.S. Environmental Protection Agency (EPA) presently defines a safe upper limit for dietary mercury exposure at 0.1μg/kg of body weight per day (2), a reference dose recently confirmed by the National Academy of Science. Maternal exposures above this level are thought to pose increasing risks to fetal brain development. This is disturbing since a woman of reproductive age can exceed this limit by eating as little as 1.5 ounces of swordfish or 7 ounces of tuna per week (based on average mercury concentrations of 1.0 and 0.2 μg mercury per gram of fish, respectively). A 20-kg child exceeds the current safe consumption limit by eating a mere half-ounce of swordfish per week or 2.5 ounces of tuna per week (3, 4). Using these values, recent methylmercury exposure assessments suggest that 7% of U.S. women of reproductive age, as well as 20% of 3–6-year-old children, exceed the safe exposure limit (5, 6). These values led the National Academy of Science to make the disturbing estimate that over 60,000 U.S. children are born each year at risk for learning and other disabilities because of prenatal methylmercury (3). At the Senate Committee on Environment and Public Works meeting during the week of September 28, 2003,

it was suggested that the reference dose (Rfd) should be lowered to 0.061µg/kg, thereby increasing the apparent number of U.S. infants at risk to Hg exposure to 300,000.

As a result of these reports, fish consumption advisories for Hg have been issued for more than 79,000 lakes, including all the Great Lakes, and more than 485,000 miles of rivers. A total of more than 2600 advisories have been issued in 49 states, the District of Columbia, and the U.S. Territory of American Samoa (7). Although adults can experience neurological effects when exposed to high concentrations of methylmercury, these advisories have mainly arisen because of the increasing concerns regarding methylmercury's effects in the developing nervous systems of unborn and growing children. Alarmingly, while the placental barrier can stop many toxic elements, methylmercury is an exception in that it not only crosses the placenta, it accumulates at higher concentrations on the fetal side than on the maternal. Worsening the situation for the developing fetus, mercury also crosses the blood–brain barrier and exhibits long-term retention once it gets across. These factors exacerbate mercury's neurotoxicity and conspire to intensify the pathologic effects in this most important and most vulnerable of the body's tissues. Destruction of an early generation of brain cells will naturally preclude development of further generations of cells, constraining development of brain and nerve tissues. While these are the expected consequences from high doses of mercury exposure, recent research suggests natural processes may be protecting us from moderate mercury exposures.

It is well recognized that mercury and sulfur bind together to form complexes. This binding property is the basis of chelating therapy used as a treatment in cases of acute mercury poisoning. The complexes between mercury and selenium are less generally known, but of much higher affinity. Physiologically, sulfur is far more abundant than selenium, yet because of selenium's higher affinity, mercury selectively binds with selenium to form insoluble mercury

selenides which are retained in brain cells (8, 9). This interaction has been assumed to be a "protective" effect whereby supplemental selenium complexes the mercury and prevents death in animals fed otherwise toxic amounts of mercury (10, 11). However, the understanding of the protective effect may actually be backwards. Instead, mercury's propensity for selenium sequestration in brain and endocrine tissues may inhibit formation of essential Se-dependent proteins (selenoproteins). If this is the case, ensuring adequate levels of selenium may reduce the risks associated with mercury exposure.

Selenoproteins have numerous recognized enzymatic functions, and there are over 20 selenoproteins whose functions remain uncharacterized. Selenoprotein activities may be especially important in brain, pituitary, and thyroid tissues since it is virtually impossible to deplete the selenium in these tissues, even after feeding selenium-deficient diets for many generations. Consequently, any element that can enter the brain and disrupt selenoprotein synthesis in these tissues will accomplish what multigenerational selenium deficiency cannot. Mercury not only has the ability to cross the blood–brain barrier, but its high selenium affinity enables it to specifically sequester the brain's selenium by forming insoluble mercury selenides, thereby diminishing selenoprotein synthesis in these tissues.

The health risks of methylmercury exposure may then vary in response to individual and regional differences in selenium intake. Selenium is highly variable, abundant in soils of one area and dangerously low in regions only miles away. Regional variances in geologic distributions of selenium will influence the amounts in foods, predisposing for or protecting against potential risks from mercury exposure. Furthermore, differences in relative quantities and quality of food choices can result in individual differences in selenium status. Consumption of foods low in selenium compromises selenium stores and may enhance the risk of shortfalls in selenoenzyme

synthesis upon mercury exposure. Thus, studying the pathology of mercury toxicity may require a more insightful question than simply, “How much mercury is consumed?” The more appropriate question may be, “Is a sufficient amount of free selenium available in the cell to create the necessary selenoenzymes or is too much of the selenium lost through binding to mercury?” The sensitivity to mercury-induced neurotoxicity may be the result of the balance of the relative amounts of mercury and selenium acting in this biological equation. Therefore, further research is needed in order to establish the true risks of moderate mercury exposure.

Simply defining the amount of mercury present in the environment or in our food sources may be an insufficient indication of the risk associated with mercury exposure without a concurrent assessment of selenium status. It is possible that people living in low-selenium areas of the world may be at greater risk from mercury exposure than populations living in regions with higher selenium status. Increasing our understanding the effects of mercury on selenium physiology and the influence of selenium status on robustness to mercury exposure are important issues to address. We need to establish the effectiveness of selenium in protecting against methylmercury exposure and better understand the effects of methylmercury on normal selenium metabolism.

Selenium's involvement in the mercury cycle is apparent throughout source, transport, biogeochemical exposure, bioavailability, toxicological consequences, and remediation. Further research in these areas will provide valuable information that may have important implications for industries that emit mercury, as well as sport and commercial fisheries, farmers, ranchers, legislators, and policy makers. Likewise, research addressing mercury's influence on selenium physiology will provide valuable information for nutritional and medical communities involved in research and public health, especially those involved in child health and development.

The determination of risks from chemical exposures is a major issue for regulators and lawmakers. Because of limited knowledge of the physiological effects of mercury, EPA was forced to establish its 0.1-µg/kg/day reference dose based on extrapolated assumptions. Therefore, enhanced awareness of the importance of selenium status in relation to mercury exposure will provide policy makers with a more complete understanding of the risks involved and enable them to make better-informed decisions.

This EERC proposal details a proposed multiclient study of mercury's effect on selenium-dependent physiology. We will resolve important questions regarding the effects of methylmercury on normal selenium physiology. The goal of this work is to study the impact of mercury exposure on selenium availability and selenium-dependent activities. We propose to analyze this interaction at the organism, tissue, cellular, protein, and molecular levels.

QUALIFICATIONS

Dr. Nicholas V.C. Ralston worked for over a decade at the Grand Forks Human Nutrition Research Center (GFHNRC) before starting training at Mayo Medical Center in biomedical research biochemistry. His research there examined the etiology of byssinosis, a chronic inflammatory lung condition. His major contribution to this field was defining new molecular mechanisms of arachidonic acid release in pathologic inflammation. His work at Bowman Gray Medical School at Wake Forest University focused on Bis(monoacyl)glycerol phosphate, a unique phospholipid resistant to phospholipase activity in the lysosomes and an important source of arachidonic acid. Returning to GFHNRC in 1998, Dr. Ralston developed the capillary electrophoresis chromatography method for quantitating boron binding to biological molecules, identifying new high-affinity boron-binding species, S-adenosylmethionine and the family of diadenosine polyphosphates. Studies of the distribution of radiolabeled selenoproteins and

selenomolecules revealed a previously unsuspected high abundance of low molecular weight selenomolecules present in brain tissues. Another project examining the relationship between selenium and inflammation resulted in recognition of new aspects of the inflammatory process that are modulated by selenium status. Since joining EERC, he has been involved in evaluating potential human health effects and risks resulting from environmental exposure to toxic metals including mercury and nickel. He has four EPA-sponsored projects currently under way as part of the Center for Air Toxic Metals® (CATM®) that are investigating basic aspects of the mercury–selenium interaction, including collaborative work on assessing the selenium status of mercury exposed mother–child pairs in the Seychelles Islands.

Dr. Laura J. Raymond graduated from the University of North Dakota in August 2002, with a Ph.D. in Biochemistry and Molecular Biology with a cognate emphasis in nutritional metabolism. Dr. Raymond's research examines biochemical and analytical approaches involved in evaluating potential human health effects and risks resulting from environmental exposure to air, water, and food toxins. Her principal areas of interest and expertise include evaluating the effects of mercury exposure in selenium-dependent physiology; analyzing the effects of environmental toxins and particulates at the biochemical and molecular levels; and the impacts of pollutants on health and physiological processes as well as strategies for prevention, protection, and remediation. Prior to joining the EERC, her predoctoral research was completed at GFHNRC studying the influence of copper status on free radical physiology and pathophysiology at the molecular level. Her research in HL-60 cells indicates copper deficiency reduces the function of cytochrome-c oxidase, which in turn causes an increase in reactive oxygen species production. These toxic species affect the cell system through cell signaling mechanisms and/or through oxidative damage. Additionally, under copper-deficient conditions,

HL-60 cell apoptosis was initiated when certain antioxidants were added to the growing media. This was not seen in copper-adequate cells. These opposing effects of antioxidants have not been previously reported and may lead to novel areas of research in free radical signaling, antioxidant mechanisms, and understanding apoptosis. Prior to graduate school, Dr. Raymond served as a medic in the United States Air Force (USAF) and USAF Reserve and received an undergraduate degree in Microbiology. She has four EPA-sponsored projects currently under way as part of CATM that are investigating basic aspects of the mercury–selenium interaction including collaborative work on assessing the selenium status of mercury-exposed mother–child pairs in the Seychelles Islands.

Dr. Steve Benson (Ph.D. Fuel Science, the Pennsylvania State University) will be the Senior Manager for the project responsible for overseeing program progress and communication with stakeholders. Dr. Benson has been a researcher at the EERC for the past 20 years, holding the positions of Associate Director for Research, Senior Research Manager of the Fuels and Materials Science Group, Research Supervisor and Research Chemist for the U.S. Department of Energy (DOE) Grand Forks Energy Technology Center, and the Director for CATM. Dr. Benson is a member of several professional organizations and has been the technical coordinator, chairman, or cochairman for several national and international conferences.

VALUE TO NORTH DAKOTA

North Dakota power utilities burn coal fuels to drive their electricity generator plants, resulting in release of elemental mercury into the atmosphere. On December 15, 2003, EPA proposed regulation to reduce mercury emissions from electric power utilities. Following public comment, EPA will promulgate a final rule by December 2004, with full implementation and compliance scheduled as early as December 2007. These regulatory mandates are in response to

mounting public concern regarding perceived risks of mercury exposure from fish consumption. However, increasingly strict regulatory policies may not be justified if natural mechanisms of protection against mercury exposure such as normal dietary selenium are sufficient. Therefore, enhancing our understanding of the effects of mercury on selenium physiology and the influence of selenium status on robustness to mercury exposure are important issues to address. The results of these studies will improve understanding of the relationship between selenium and mercury and provide important information to regulatory agencies useful in making their policy decisions.

If normal dietary intakes of selenium are found to be sufficient to protect against potential negative consequences of moderate mercury exposure, fish advisories in North Dakota may eventually be relaxed, enhancing the draw and appeal of sport fishing in our lakes and reservoirs. Since North Dakota farmers produce grains and beef that have higher selenium contents than those of many other regions, local agriculture will benefit from enhanced consumer appreciation for the health benefits accompanying the selenium present in their food products.

The results of this research effort will be published in peer-reviewed journals focusing on environmental, health, and nutritional topics. In order to further disseminate the results of this research, we will present our findings at national and international meetings.

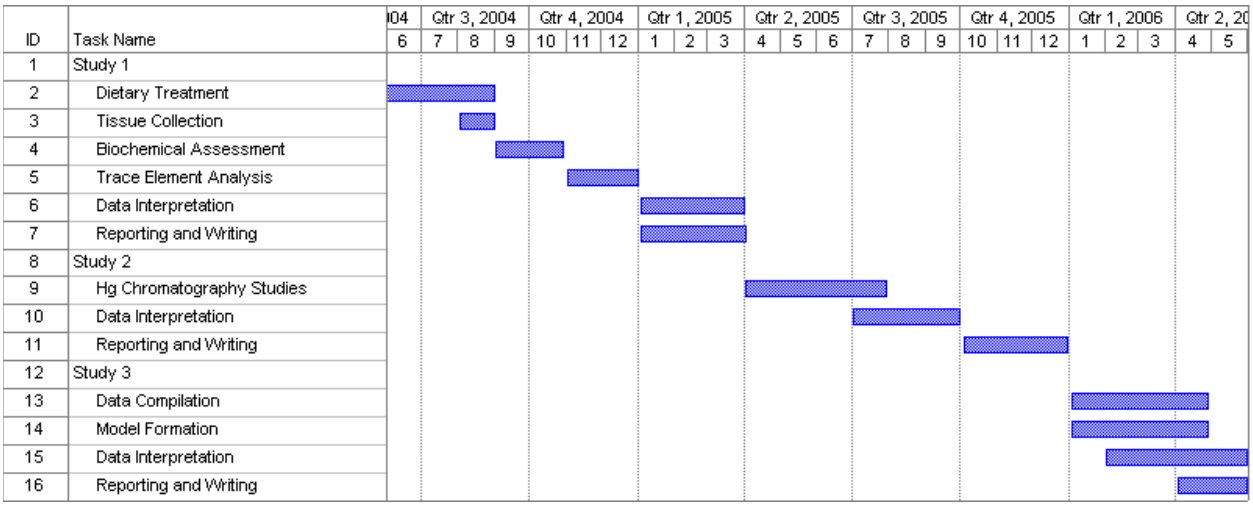
MANAGEMENT

Dr. Steven Benson will be the Senior Manager for the project responsible for overseeing program progress and communication with stakeholders. Dr. Nicholas Ralston will be Project Manager, responsible for guiding the design and performance of the scientific protocols of the study. Dr. Laura Raymond will oversee the performance and execution of the projects' animal care, sample preparation, and analysis concerns. Resumes are included in Appendix D. Further technical personnel supporting the project will be drawn from existing EERC research staff.

These staff members are highly trained and have had substantial experience with evaluation of trace metal analysis and physiology.

The Environmental Health Research Group's mission is to evaluate environmental toxicity hazards as well as prevention and remediation strategies. Facilities at the EERC are among the best in the world and include state-of-the-art instrumentation and equipment for performing analytical research studies. Aside from two specialized instruments, one for assessing motor function and a dual detector system for on-line analysis of mercury and selenium that will be funded through this proposal, all other equipment needed to complete this project is currently available. Additional information on the programs, personnel, and instrumentation at these facilities are available upon request.

TIMETABLE



BUDGET

The estimated cost of the proposed research program is $153,846 over a 24-month period. Please see Appendix E for budget details.

MATCHING FUNDS

We propose cost-share sponsors provide $50,000, the North Dakota Industrial Commission contribute $50,000, and DOE, through the EERC–DOE Jointly Sponsored Research Program, contribute approximately 35% or $53,846. Financial support from the cost-share partners is being finalized. Additional partners have expressed confidence they will join in funding the expansion of Year 2 research. When this occurs, we will increase the scope of the research studies as described in Appendix A. We will provide documentation of these support agreements prior to North Dakota Industrial Commission making an award.

Three items are required from NDIC for inclusion in our proposal to DOE:

- A formal commitment to the project. This can be a letter of commitment, a purchase order, or a signed contract.
- A biographical sketch or resume for NIDC's project manager or key technical contributor.
- A short overview of NDIC.

The EERC will submit a proposal to DOE for its approval upon receipt of NDIC's commitment and the information above.

TAX LIABILITY

The EERC is part of the University of North Dakota, a tax exempt entity.

CONFIDENTIAL INFORMATION

No confidential information is expected to result from performance of this project.

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APPENDIX A

INCREASED SCOPE RESEARCH STUDIES

INCREASED SCOPE RESEARCH STUDIES: SELENIUM PROTECTION FROM TOXIC EFFECTS OF MERCURY EXPOSURE

This study will establish the protective effects of dietary selenium against neurofunctional consequences of mercury exposure in laboratory rats fed torula yeast-based diets prepared with selenium at levels that are deficient (0.1 mM), adequate (1.0 mM), or enriched (10.0 mM) and supplemented with mercury at 50 mM or control animals fed adequate selenium with no added mercury. One hundred thirty Long Evans rats will be assigned to weight-matched groups (30 Se-deficient + Hg, 40 Se-adequate + Hg, 30 Se-rich + Hg, and 30 Se-adequate no Hg) that will be fed diets prepared with selenium and methylmercury as indicated.

The rats will be repeatedly tested throughout the study to establish and continuously monitor their motor coordination function levels. This will be achieved through the use of an instrument designed to measure motor coordination capabilities of four animals at a time, obtaining a sensitive record of this aspect of their neurological function. In these tests, rats are placed on top of a drum that rotates at precisely monitored speeds that gradually increase, forcing the rat to walk forward to maintain its balanced position. The speed of rotation escalates at a defined rate according to a program that is exactly repeated during each test cycle. When the rat is no longer able to keep up and maintain its balance, it falls approximately 10 in. onto a pad that triggers an actuator switch, recording the time that this rat was able to keep pace. This test will be repeatedly performed, and the average of three repetitions of this test will be recorded for each rat. The mean of these values for each group will be evaluated for each assessment time point so that the effect of dietary treatment on rat performance can be tracked during the course of the study. Selenium-deficient rats are expected to be far more sensitive to mercury exposure than rats fed diets with adequate selenium which will, in turn, be more sensitive to mercury than rats fed diets rich in selenium. Five members of each dietary treatment group will be terminated

at a time preceding development of impairment, five more when impairment becomes apparent, and five more when the impairment becomes pronounced. Rats fed the selenium-adequate and selenium-rich diets will continue to be functionally assessed until impairment becomes apparent in the selenium-adequate group, at which time five rats from each diet treatment group will be terminated (see diagram in Appendix B). The rats fed the selenium-rich diet may not show any neurological impairment from the mercury exposure, but their motor coordination will be tested repeatedly to monitor their motor coordination status in comparison to the other treatment groups. Parallel sets of rats in groups showing compromised motor coordination will be switched to mercury-free diets containing either low or high selenium (see Appendix B). The motor function of these sets of "recovery" rats will be compared to those maintained on mercury-containing diets.

At the time of termination, the blood, brain, pituitary, liver, kidney, and testes will be collected and analyzed for mercury and selenium content as well as selenium-dependent enzyme activity. As in Study 1, in these latter tissues, the molar distribution of mercury and selenium in the total tissue as well as the distributions in the low-molecular-weight fraction expected to accumulate residual mercury–selenomolecule complexes will be measured. Selenium concentrations in these samples will be determined using graphite furnace atomic absorption spectroscopy (GFAAS) while mercury concentrations will be determined using cold-vapor atomic absorption spectroscopy (CVAAS) by the Analytical Research Laboratory at the Energy & Environmental Research Center (EERC).

Using the same methods applied in Study 1, the mercury- and selenium-containing species in the <5kD-molecular-weight fraction will be separated and detected using a Millennium Merlin atomic fluorescence system in parallel with a Millennium Excalibur atomic fluorescence system.

We will determine the quantities and distributions of mercury arising in the <5-kD fraction of tissues from animals fed each of the forms and concentrations of selenium. The effects of selenium on mercury accumulation and distribution and selenium on mercury accumulation and distribution molecular species in total and low-molecular-weight fractions from the various tissues will be quantitatively compared. The effects of Hg and Se on neurofunctional performance and selenoenzyme activities will be compared using analysis of variation (ANOVA).

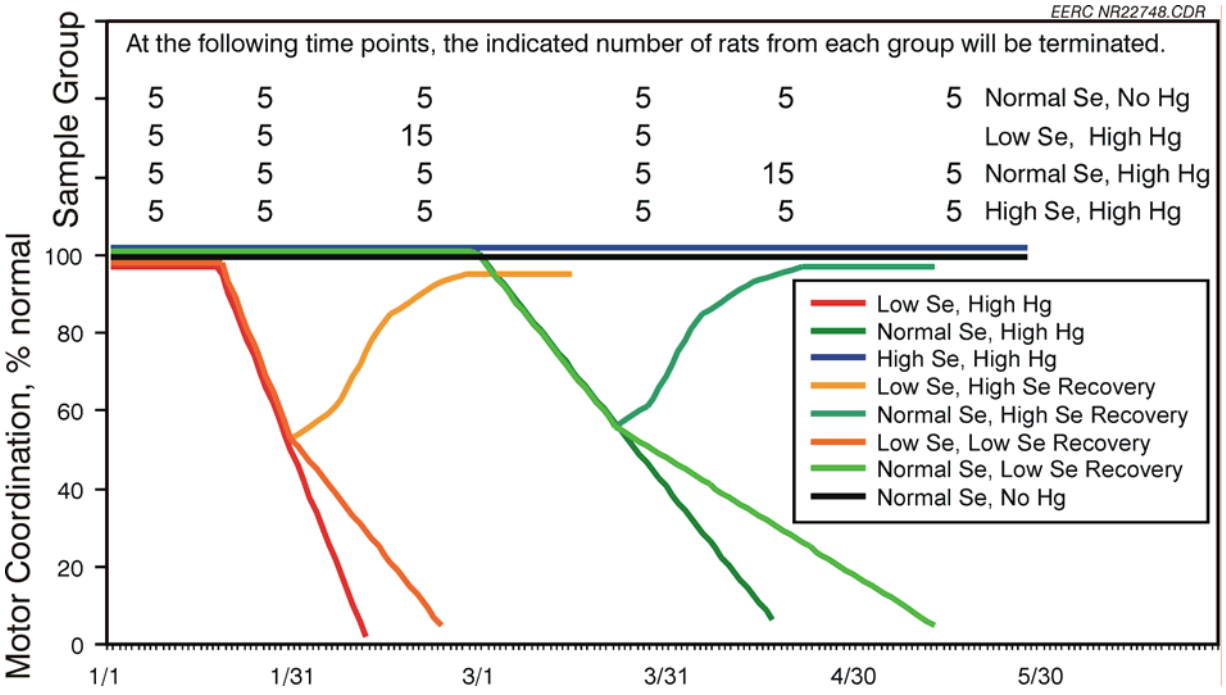

Figure A-1. Diagrammatic depiction of study.

This figure depicts the hypothetical effects of feeding diets with different mercury and selenium concentrations on motor function in rats. The projected toxic effects of mercury and protective effects of selenium depicted in this diagram are based on the authors' experience with

the interactions of these elements in preliminary animal research studies presented in Appendix C of this proposal.

Four dietary treatments will be studied. The motor performance of control rats fed diets prepared with adequate selenium and no added mercury are presented in black. The performance of these rats will set the 100% (normal) function standard for this study. The motor performance expected for rats fed diets containing mercury added at a concentration of 50 mM (10 ppm) are presented in red, green, and blue. Rats fed the selenium-deficient diets (0.1 mM Se; red) will be more sensitive to mercury exposure than rats fed diets containing normal (1.0 mM Se; green) concentrations of selenium. Rats fed selenium-rich diets (10 mM Se; blue) are expected to be even less sensitive to the effects of mercury in the diets and may not show measurable retardation of their motor function during the course of this study.

As indicated at the top of the figure, groups of animals from each of the treatment groups will be terminated at various times during the study. Once rats have been on their diets for 3 weeks, tissue samples will be collected to reflect their initial Hg–Se ratios before any Hg-dependent retardation of motor coordination function is observed. When rats in the selenium-deficient treatment group show notable diminishment in motor function, the second set of tissue samples will be collected.

At this time, the rats in the selenium-deficient group will be subdivided into three cohorts; one will continue to receive the low-selenium diet supplemented with 50 mM Hg, the other two groups will be switched to mercury-free diets that contain either low (0.1 mM)- or high (10.0 mM)-selenium concentrations. Five members of each of these subgroups (total of 15 rats) will be terminated at the next time point, which will be determined by the decline in motor capability of the rats continued on the low-Se, high-Hg diets. Rats that have been switched to a

10-mM Se diet without added Hg are expected to recover motor function to some degree, but may or may not attain complete recovery of motor function. When rats in the group fed normal selenium show diminished motor function, they will be subdivided into three subgroups in which the effects of +/− selenium recovery diets will be tested just as had been done for the Se-deficient animals. We expect the rate of decline in motor coordination to be less rapid in this group and expect a more rapid and more complete recovery of motor function in the high-Se supplemented rats.

Increased Scope Research Studies: Methylmercury Binding Affinities to Selenoproteins

Mercury has an extraordinarily high binding affinity for selenoprotein P, a plasma protein that is an abundant source of selenium because of the uniquely high number of selenocysteines present in each molecule. This is important since selenoprotein P supplies a large percentage of the selenium taken up by the brain and testes. Animals challenged with high-mercury diets may therefore experience increased mercury delivery to the brain and testes through association with this protein and may simultaneously experience diminished uptake of free selenium from this source. Other aspects of the mercury–selenium interaction seem to also involve selenoprotein P. For instance, it is interesting to note that mice that have had their gene for selenoprotein P experimentally removed show poor weight gain and loss of motor coordination. These signs are similar to those evident in mercury-intoxicated rats. Supplying additional dietary selenium to these mice protected them from these negative effects. This supports our hypothesis that mercury toxicity may induce a conditioned selenium deficiency.

Selenoprotein P will be isolated from normal plasma using chromatography techniques and purified using affinity chromatography methods. The isolated selenoprotein P will be exposed to

mercury-containing solutions and quantitatively assessed using high-performance liquid chromatography (HPLC) methods to separate free and selenoprotein P-bound mercury that will be quantitated using a Millennium Merlin detector (to measure mercury) in parallel with a Millennium Excalibur detector (to measure selenium). Measurements of mercury binding to this molecule may be complemented by parallel studies of mercury binding to synthetic peptides incorporating selenocysteine.

These binding experiments will be repeatedly performed to validate accuracy and precision of the determinations of mercury interactions with the selenocysteines of selenoprotein P and other biologically important forms of selenium.

APPENDIX B

TECHNICAL BACKGROUND

TECHNICAL BACKGROUND

Mercury is of increasing concern as a global pollutant. Because of its bioaccumulative nature and acute neurotoxicity, the International Program of Chemical Safety lists methylmercury as one of the most dangerous chemicals in the environment (1). Based on the current understanding of mercury toxicity, the U.S. Environmental Protection Agency presently defines a safe upper limit for dietary mercury exposure at 0.1 μg/kg of body weight per day (2), a reference dose (Rfd) recently confirmed by the National Academy of Science. Maternal exposures above this level are thought to pose increasing risks to fetal brain development. This is disturbing since a 135-lb woman can exceed this limit by eating as little as 1.5 ounces of swordfish or 7 ounces of tuna per week (based on average mercury concentrations of 1.0 and 0.2 μg mercury per gram of fish, respectively). A 50-lb child exceeds the current safe consumption limit by eating as little as one half-ounce serving of swordfish or 2.5 ounces of tuna per week (3, 4). Recent exposure assessments suggest that 20% of 3–6-year-old children as well as 7% of U.S. women of reproductive age exceed the recommended safe exposure limit for methylmercury (5, 6). Using these values, the National Academy of Science estimated that over 60,000 U.S. children are born each year at risk for learning and other disabilities (3). At the Senate Committee on Environment and Public Works meeting during the week of September 28, 2003, it was suggested that the Rfd should be lowered to 0.06 μg/kg. Such a change would increase the perceived number of U.S. infants at risk from mercury exposure to 300,000. As a result of these concerns, fish consumption advisories for mercury have been issued for more than 79,000 lakes, including all of the Great Lakes, and more than 485,000 miles of rivers. A total of more than 2600 advisories have been issued in 49 states (7).

Although adults can experience neurological consequences when chronically exposed to high concentrations of methylmercury, these advisories have arisen because of concerns regarding methylmercury's effects in the developing nervous systems of unborn and growing children. While the placental barrier can stop many toxic elements, methylmercury not only crosses the placenta, it accumulates at higher concentrations on the fetal side. Methylmercury also readily crosses the blood–brain barrier and exhibits long-term retention once it enters the brain. These factors exacerbate methylmercury's neurotoxicity and intensify its pathologic effects in this most important and vulnerable of the body's tissues. Effects are time-dependent since the destruction of an earlier generation of brain cells impairs development of brain and nerve tissues far more than would loss of an equal number of cells in later generations. While these are the known consequences of acute high-dose exposure to methylmercury, the effects of chronic low-dose exposures are undetermined.

There are major differences in the observations and conclusions of the methylmercury exposure studies that have been done in the Faroes and Seychelles Islands (8, 9). While the Faroe Island researchers reported neurological effects in children exposed to mercury in the womb, the Seychelles mercury study found no adverse health effects from prenatal mercury exposure, even at levels of exposure 10–20 times higher than in the United States. Instead, the Seychelles study found maternal fish consumption correlated with an improved neurodevelopmental outcome in some functional indices. Although appearing contradictory in their findings, both studies were well conducted and scientifically credible. The discrepancies between their observations and conclusions may be due to dietary differences in the study populations. Since selenium status can mitigate the consequences of mercury exposure, it is possible qualitative differences in background food sources in the two locations may be a factor.

Although sulfur's high binding affinity with mercury is more generally recognized, the affinities of mercury-selenium complexes are far greater. Sulfur is 10–100,000 times more abundant in physiological matrices than selenium, yet because of selenium's higher affinity, mercury selectively binds with selenium to form insoluble mercury selenides (10, 11). This interaction has been assumed to provide a “protective” effect whereby supplemental selenium forms complexes with mercury and prevents death in animals fed otherwise toxic amounts of mercury (12, 13). The ability of selenium compounds to decrease the toxicity of mercury has been established in all investigated species of mammals, birds, and fish.

Research on the interaction between selenium and mercury has a relatively long history. Watanabe noted that in the 1950s, it was recognized that tissues from Minamata residents that had high concentrations of mercury also had unusually high selenium concentrations (14). In 1967, Parizek and Ostadalova reported on the alleviation of the lethal toxicity of mercuric chloride by sodium selenite simultaneously administered to rats (15). In 1972, Ganther et al. showed the mitigating effect of sodium selenite on the toxicity of methylmercury. Their results indicated sodium selenite reduced methylmercury-induced mortality and alleviated the suppression of weight gain in rats (16). In 1975, Kosta et al. noted mercury and selenium had coaccumulated in autopsy tissues of mercury miners (17). As a result of these findings, extensive research has been done regarding the interactions of these two elements. Recent investigations continue to support the importance of mercury's effects on selenium-dependent metabolism.

Ironically, until approximately 45 years ago, selenium was known only as a poison itself. It is now known that selenium is essential for the normal function of 20–30 enzymes in the body. Selenium can act as a growth factor; has powerful antioxidant and anticancer properties; and is involved in thyroid hormone homeostasis, immunity, and fertility. Although still omitted from

many biochemistry textbooks, two of the 22 physiologically significant amino acids are distinguished by their possession of selenium: selenomethionine and selenocysteine.

Selenomethionine is physiologically equivalent to methionine and is regarded as an unregulated storage compartment for selenium. In contrast, selenocysteine is tightly regulated and specifically incorporated into numerous proteins that perform significant biological functions. More than two dozen selenoproteins occur in tissue specific distribution in animal cells. Selenoprotein activities may be especially important in the brain, pituitary, and thyroid since these tissues are virtually impossible to deplete of their selenium. Selenium depletion studies conducted over six generations in rats led to a drastic decrease of selenium concentrations in their liver, skeletal muscle, and blood to levels below 1% of normal brain. Meanwhile, brain tissues in these rats retained selenium at a concentration ~60% of that found in control animals. Further studies showed rats maintained same level of retention through 16 generations of being fed selenium-deficient diets (18).

Although brain selenium concentrations in normal rats could not be reduced to less than 60% of normal, Burk found that feeding diets containing less than 0.1 ppm selenium to selenoprotein P knockout mice reduced their brain selenium concentrations to 43% of normal, the lowest brain selenium concentration achieved in any experimental animal model (19). While rats with brain selenium's at 60% of normal were asymptomatic, these mice demonstrated pronounced loss of motor coordination that could be corrected by feeding them diets containing selenium at 2 mg/kg diet. Motor coordination was restored when their brain selenium content was replenished. Further indication of selenium's essential role in supporting basic life processes is given by the report of Nishimura et al., showing that the total disruption of selenoprotein synthesis in mice, achieved by knocking out the selenocysteinyl-tRNA gene, resulted in early

embryonal lethality (20). Selenium's role in maintaining normal brain function is further indicated by the discovery that selenium deficiency accelerates the turnover of monoamines in regions of the brain such as the hippocampus and substantia nigra in adult rats (21). These studies indicate the importance of selenium to normal brain functioning and support the concept of tight regulation of this element in the brain.

Accordingly, if an agent with high affinity for selenium were able to enter the brain, sequester, and thus divert the cell's selenium from its normal physiological roles, effects similar to severe selenium deficiency might be expected. Methylmercury not only has the ability to cross the blood–brain barrier in significant mass quantities when it is abundant in foods, but its exceptionally high affinity for selenium appear likely to enable it to specifically sequester intracellular selenium in brain tissues and diminish their selenoprotein synthesis. The affinity constant for selenocysteine's selenium and mercury is ~10^{-22}, and the free selenides that form during each cycle of selenocysteine synthesis have an exceptionally high affinity constant for mercury: 10^{-50}. Mercury selenide precipitates have extremely low solubility, ranging from 10^{-58} to 10^{-65} (22); thus they are thought to be metabolically inert (23). Thus mercury's propensity for selenium sequestration in brain and endocrine tissues may inhibit formation of selenoproteins. It is possible selenium's "protective effect" against toxic amounts of mercury may reflect the mass action effect of supplemental selenium supporting selenoenzyme synthesis when the mercury would otherwise have overwhelmingly diverted all available selenium into formation of insoluble mercury–selenide complexes (see Figure B-1).

Investigations on humans (24–27) and animals (28–32) have shown that at a higher mercury burden, the molar ratio of selenium and mercury in tissues tends to approach a 1:1 stoichiometry (33). The nature of the interaction between mercury and selenium seems to depend

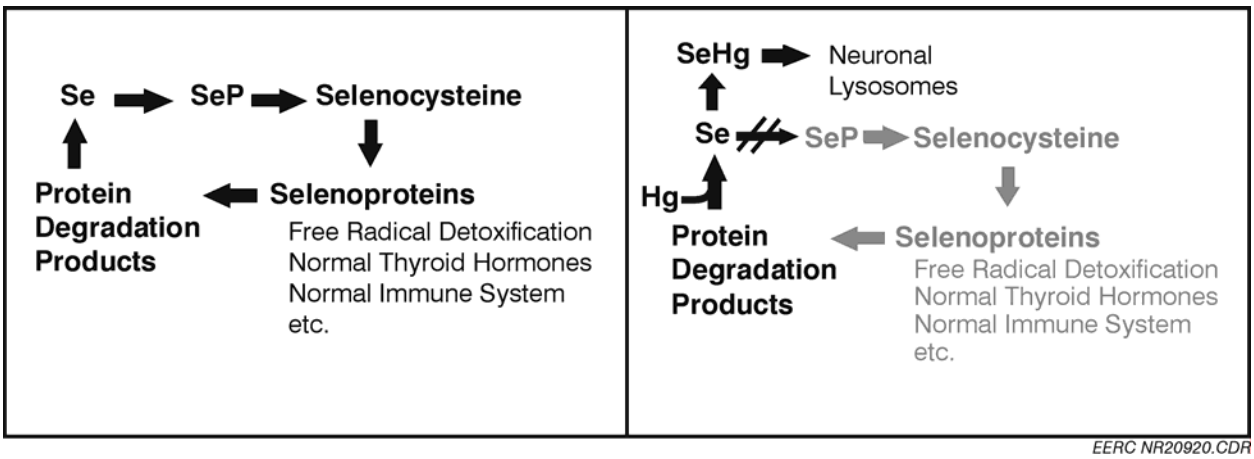

Figure B-1. Effect of mercury on selenium physiology.

on the chemical forms of both elements. The acute interaction between toxic doses of inorganic mercury and toxic doses of selenite in adult rodents has been thoroughly examined and is described as a mutual alleviation of toxicity through formation of inert Hg–Se complexes (34).

In a study involving monkeys chronically exposed to methylmercury, the concentrations of selenium and inorganic mercury in the brain were correlated, while those of selenium and total mercury were not. The authors speculated that Se–Hg complexes are formed in the brain and that methylmercury may induce a local selenium deficiency in the brain by diverting selenium from selenoprotein synthesis to formation of the complex (35).

Studies indicate that when sodium selenite is coadministered with methylmercury, the fetotoxicity, neurotoxicity, and developmental toxicity of methylmercury is alleviated, and mercury toxicity is enhanced in selenium-deficient animals. Selenium-deficient rodents are more susceptible to the prenatal toxicity of methylmercury, and it is noteworthy that exposure to mercury reduced the activity of the selenoprotein glutathione peroxidase in the fetal/neonatal brain without affecting the level of selenium in the fetal liver (36). Injections of HgCl into

pregnant rats increased the retention of a tracer dose of selenium in maternal blood and liver but decreased selenium in fetal blood (37).

In mice prenatally exposed to methylmercury, the liver concentration of selenium increased while its selenium-dependent glutathione peroxidase activity was reduced (38). It has also been shown that maternal exposure to methylmercury decreased both selenium concentration and glutathione peroxidase activity in the brain of neonatal mice and also diminished the activities of selenium-dependent iodothyronine deiodinases (39). Watanabe reported that mercury exposure of selenium-deficient perinatal mice resulted in retarded neurobehavioral development and persistent learning disabilities. He postulated that in utero selenium deficiency and methylmercury exposure affect the neurobehavioral function of the offspring in an additive manner. He examined the effects of prenatal methylmercury exposure on several neurobehavioral end points using groups of mice given various dietary amounts of selenium. All toxicity effects were exacerbated by perinatal selenium deficiency. In addition, to determine whether methylmercury exposure induces local selenium deficiency in the fetal brain, selenium concentrations and the activity of glutathione peroxidase were measured in the neonatal brain and other organs. Their results showed that methylmercury affects the metabolism of selenium in the fetal brain. Although the dietary level of selenium did not affect the mercury concentration in the fetal brain, the selenium concentration and the activity of glutathione peroxidase were severely depressed by methylmercury in the neural tissue (40).

Additionally, when rodents are depleted of selenium perinatally, the thyroid hormone economy of the fetus is disturbed (41). Thyroid hormones are essential for normal neurological development, and if thyroid hormone regulation is disrupted at vulnerable periods of development, irreversible neurological damage can result. Iodothyronine deiodinases are

selenoproteins that regulate the tissue levels of thyroid hormones. Therefore, severe depletions in available selenium may be also be detrimental to the developing brain as a result of diminished iodothyronine deiodinase activity. Together with observations that selenium deficiency exerts adverse effects on neurobehavioral development and on brain neurotransmission (42–45), these results suggest that the neurobehavioral toxicity of methylmercury may result from a deficiency of available selenium in brain tissues. Thus it is possible that methylmercury impairs selenium availability and causes a functional, local selenium deficiency in which selenoprotein synthesis is compromised while the concentration of selenium in the tissues might remain unaffected.

Unfortunately, the bioavailability studies examining selenium–methylmercury interactions are limited. Most studies have applied physiologically inappropriate doses of methylmercury and in many studies, selenium and methylmercury have been injected instead of supplied in food (46–51). Also, in most cases, little or no attention has been given to the selenium status of the experimental animal used. Studies indicate that when mercury and selenium are fed together to pigs, rats, and chickens, neither can be found in the body at anticipated levels (52–53). It is suggested that mercury therefore reduces the ability of these animals to absorb selenium (54). Glynn et al. analyzed the influence of sodium selenite supplementation on the absorption, distribution, and elimination of mercury in mice orally exposed to a nontoxic dose of methylmercury. Their results indicated that selenium treatment of methylmercury-exposed mice might have had a positive effect on the health of the animals by decreasing the total body burden of methylmercury. The elimination rate of ^{203}Hg from the whole body of methyl ^{203}Hg-exposed male mice increased with increasing selenium status. The elimination rate was increased without any changes in intestinal absorption of ^{203}Hg. They suggested that mice with a high selenium status attain a lower whole-body burden of mercury than animals with normal selenium status

during chronic exposure to methylmercury. The increased elimination of ^{203}Hg did not result in lower ^{203}Hg levels in the main target organ of mercury toxicity, the brain, suggesting selenium might exert its effects through mercury elimination from other tissues of the animals (55) or through a direct mechanism such as those depicted in Figure B-1. To date, there have been no bioavailability studies assessing the mercury–selenium interaction using natural food forms of selenium. Thus the impact of these natural forms of selenium on methylmercury absorption, retention, and distribution remain to be defined. Similarly, the influence of dietary methylmercury consumption on the absorption, retention, and distribution of these natural forms of selenium needs to be elucidated.

Methylmercury concentrations in fish flesh rise with age, but it appears that the selenium levels in fish keep pace to provide protection against mercury toxicity (for a review of selenium and mercury interactions in fish and marine animals, see Lourdes et al. [56]). Friedman et al. studied the protective effect of freeze-dried swordfish on methylmercury toxicity in rats. The rats that were experimentally administered methylmercury and fed a swordfish diet showed no signs of neurotoxic effects characteristic of mercury poisoning, while rats fed methylmercury in addition to other nonfish meals did. Analysis showed the molar concentrations of selenium in the swordfish tended to be at least twice as high as the mercury concentrations. The authors suggested that the excess selenium protected the rats from the negative consequences otherwise associated with the methylmercury that was administered (57).

Additionally, several studies indicate selenium diminishes the bioaccumulation of mercury in fish (58–60). Paulsson and Lindbergh reported a 75%–85% reduction in mercury levels of fish measured over a 3-year period after selenium supplementation to lake waters in Sweden (61). Southworth and Peterson reported a steady increase in mercury concentrations in fish following

the elimination of selenium-rich discharges of fly ash to Rogers Quarry in Tennessee in 1989. As aqueous selenium concentrations decreased from 25 to < 2 ng/L, mean selenium concentrations in bass declined from 3 to 1 mg/kg over the first 5 years and remained at 1–1.5 mg/kg for the last 3 years of the study. During this time, mean mercury concentrations in bass rose from 0.02 to 0.61 mg/kg (62– 63). Studies such as these confirm the importance of selenium consideration in providing mercury exposure management.

Therefore, the health risks of methylmercury exposure may vary in response to individual and regional differences in selenium intake. The geological distribution of selenium can be highly variable within regions, abundant in soils of one area and dangerously low in regions only miles away. Regional, national, and international variances influence the amounts of selenium present in foods that may predispose for or protect against consequences of mercury exposure. The central region of the United States has robust soil selenium levels (64), and foods produced from this region are selenium rich. As a result of the centralized food-distribution system, the U.S. population is supplied foods produced from these selenium-rich regions and, therefore, has a healthy selenium status. However, in other regions of the world, the selenium status can vary dramatically.

In summary, simply defining the amount of mercury present in the environment or in our food sources may be an insufficient indication of the risk associated with mercury exposure without a concurrent assessment of selenium status. It is possible that people living in low-selenium areas of the world may be at greater risk from mercury exposure than populations living in regions with higher selenium status. Increasing our understanding the effects of mercury on selenium metabolism and the influence of selenium status on robustness to mercury exposure are important issues to address. We need to establish the effectiveness of selenium in protecting

against methylmercury exposure and better understand the effects of methylmercury on normal selenium metabolism.

Selenium's involvement in the mercury cycle is apparent throughout source, transport, biogeochemical exposure, bioavailability, toxicological consequences, and remediation. Further research in these areas will provide valuable information that may have important implications for industries that emit mercury, as well as sport and commercial fisheries, farmers, ranchers, legislators, and policy makers. Likewise, research addressing mercury's influence on selenium metabolism will provide valuable information for nutritional and medical communities involved in research and public health, especially those involved in child health and development.

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APPENDIX C

PRELIMINARY DATA ON MERCURY–SELINIUM INTERACTIONS

The Environmental Health Research Group recently conducted a study of the interactions between dietary mercury and selenium in which the influence of dietary selenium in protection against mercury toxicity was clearly apparent. The three figures depicted on this and the following pages show the weights of rats fed diets prepared at selenium-deficient, -adequate, or -rich concentrations and supplemented with increasing concentrations of mercury.

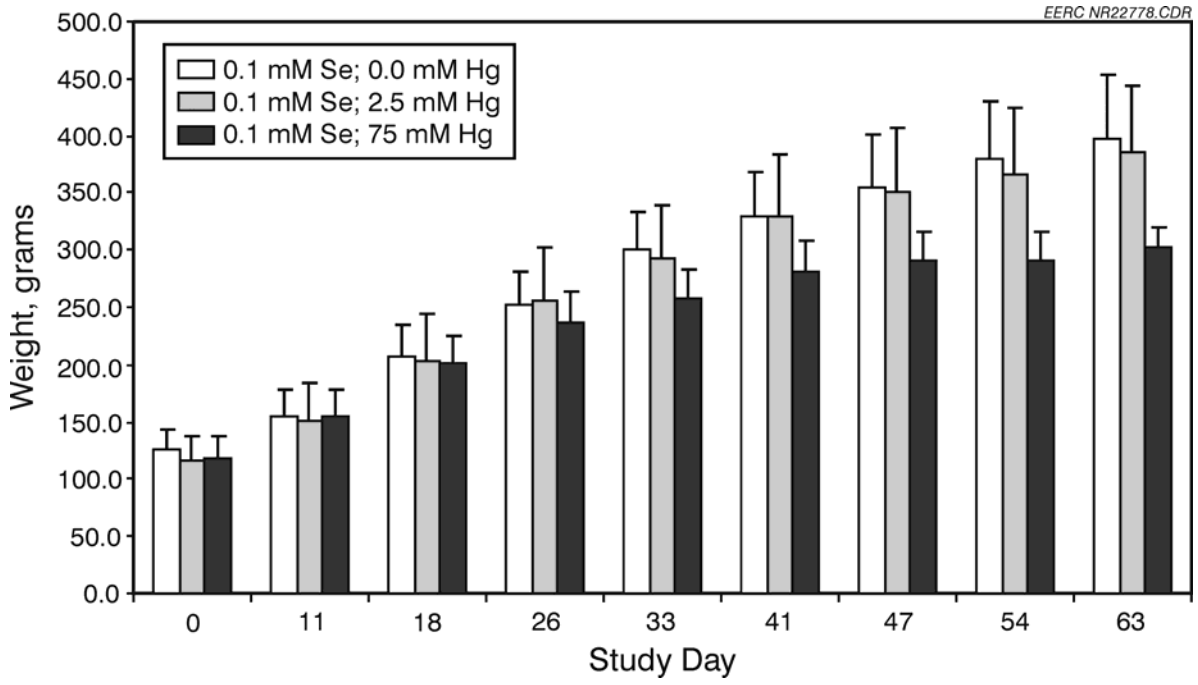

Figure C-1. Selenium-deficient rats are sensitive to mercury exposure.

The means and standard deviations (seven rats per group) of weights of growing rats fed a selenium-deficient diet supplemented with increasing concentrations of mercury are depicted in this figure. The rats were fed diets containing ~0.1 mM (0.01 ppm) selenium and either 0, 2.5, or 75 mM (0, 0.5, or 15 ppm) mercury and weighed at ~weekly intervals during the 9-week study. Weight gain among rats fed 75-mM Hg diets was significantly diminished from Day 33 on in these selenium-deficient rats.

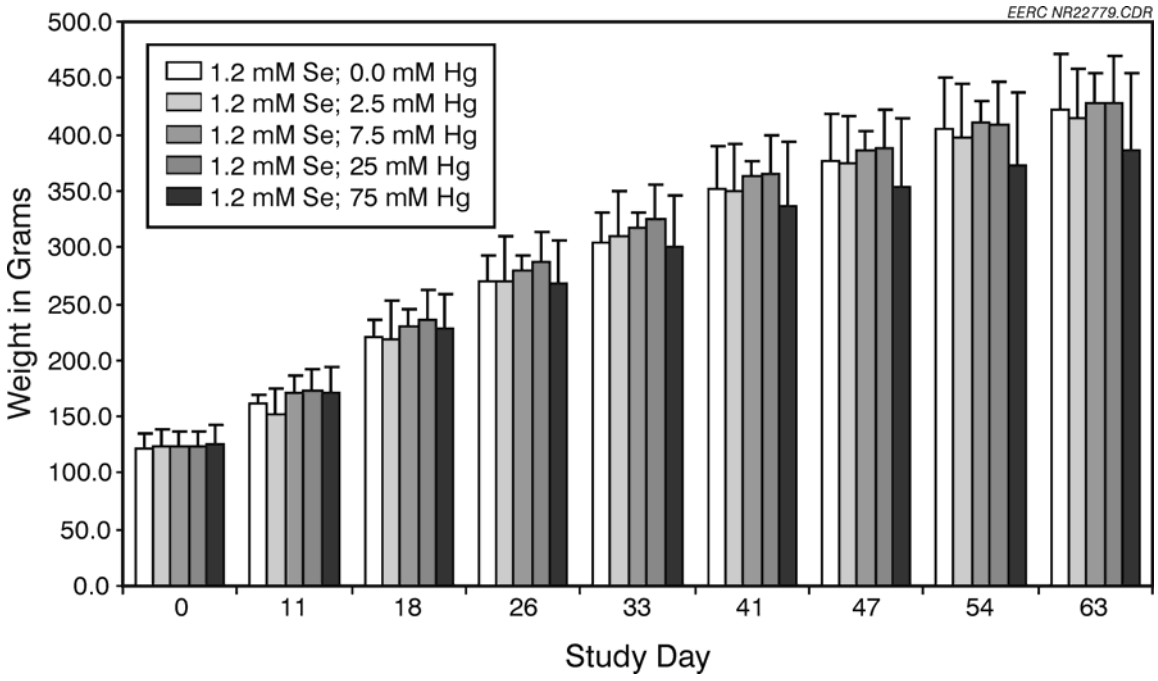

Figure C-2. Rats fed adequate dietary selenium are resistant to mercury toxicity.

The means and standard deviations (six rats per group) of weights of growing rats fed a selenium-adequate diet supplemented with increasing concentrations of mercury are depicted in this figure. The rats were fed diets containing ~1 mM (0.1 ppm) selenium and either 0, 2.5, 7.5, 25, or 75 mM (0, 0.5, 1.5, 5.0 or 15 ppm) mercury and weighed at ~weekly intervals during the study. Weight gain among rats fed the 75-mM Hg diets tended to be less, but was not significantly diminished during this 9-week study.

The means and standard deviations (six rats per group) of weights of growing rats fed a selenium-rich diet supplemented with increasing concentrations of mercury are depicted in this figure. The rats were fed diets containing ~25 mM (2.0 ppm) selenium and either 0, 2.5, or 75 mM (0, 0.5, or 15 ppm) mercury and weighed at ~weekly intervals during the 9-week study. Weight gain among rats fed 75-mM Hg diets was unaffected in rats fed selenium-rich diets.

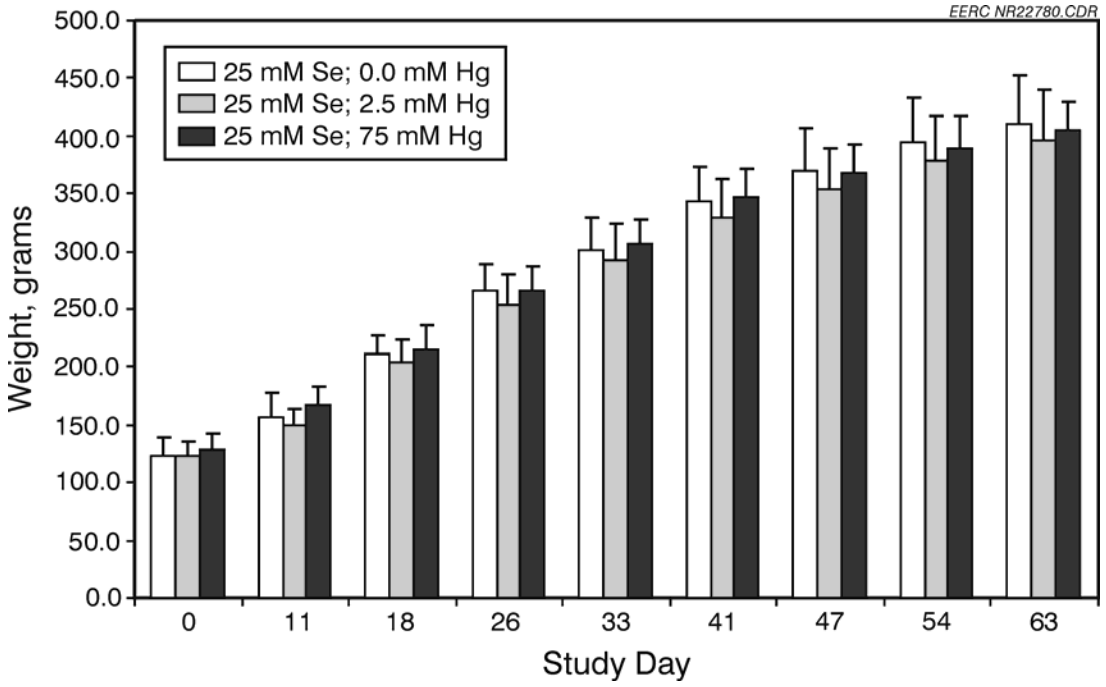

Figure C-3. Rats fed supplemental selenium show no mercury toxicity.

APPENDIX D

RESUMES

DR. NICHOLAS V.C. RALSTON
Research Scientist
Energy & Environmental Research Center (EERC)
University of North Dakota (UND)
PO Box 9018, Grand Forks, ND 58202-9018 USA
Phone (701) 777-5066 Fax (701) 777-5181
E-Mail: nralston@undeerc.org

Principal Areas of Expertise

Biochemistry, physiology, and analytical approaches to quantitative assessment of immune response and inflammation at the molecular and cellular level. Program areas include the pathophysiology of pollutant exposures as well as strategies for prevention, protection, and remediation. The physiological roles of trace elements in human health, particularly in countering toxic agent exposures, are a further area of acute interest.

Qualifications

Ph.D., Biomedical Research Biochemistry, Mayo Medical Center, 1995.
B.S., Biology Composite, Mayville State University, 1978.

Professional Experience

2002 – Research Scientist, EERC, UND. Primary responsibility involves the study of pathophysiological influences of pollutant exposures in disease processes. Inhaled pollutants cause transient changes in cellular signal responses, particularly in the local high-concentration regions immediately adjacent to the particulates, thus contributing to initiation and exacerbation of respiratory inflammation that can proceed to cause acute or chronic changes in pulmonary function. Meanwhile, consumed pollutants generally demonstrate extended biological residence and initiate chronic pathologies sometimes difficult to correlate with exposure. His current research examines the role and mechanisms of selenium-dependent enzymes and low-molecular-weight species in biochemical protection against mercury's neurotoxic effects.

1998 – 2002 Research Biochemist. Human Nutrition Research Center (HNRC), U.S. Department of Agriculture (USDA), Grand Forks, North Dakota. Responsibilities included studying and recommending assays of immune parameters to include in an international collaborative study to be performed in China. To test and develop these assays, he initiated a series of animal and cell culture models of selenium-dependent processes in acute and chronic inflammation. He was also instrumental in organizing and developing several aspects of molecular biology research plans related to this study and general HNRC programs and was responsible for organizing instrument comparisons for selecting gene arrays and RT-PCR equipment. He introduced models of acute and chronic inflammation to the laboratory's studies and discovered low-molecular-weight selenomolecules are abundant in brain tissue, a previously unsuspected feature of selenium metabolism. He created the capillary electrophoresis method of detecting and

quantifying discrete boron complexes in biological samples, the first and only method with this unique capability.

1996 – 1998 Research Fellow, Bowman Gray Medical School, Wake Forest University, Winston-Salem, North Carolina. Studied and detailed the biosynthetic pathway for cellular production of sn-1-sn-1' Bis(monoacyl-glycerol)phosphate (BMP), a unique phospholipid comprising up to 50% of the lipid membrane in active lysosomes and ~20% of the total phospholipid present in alveolar macrophages. This molecule is an important source of arachidonic acid, the precursor for numerous inflammation regulatory eicosanoids. Applying the full spectrum of chromatography methods and developing novel applications of stereochemical and mass spectroscopy analysis, he determined the precursor–product reaction sequence of BMP biosynthesis in macrophages and monocytic cell lines. He also discovered the uptake and incorporation of docosahexaenoic acid (C22:6) into RAW 264.7 cell BMP is extremely rapid and highly selective: ~90% of added C22:6 is esterified into cellular BMP within seconds after cell contact. This work is in its final stages of preparation for submission to *Biochimica et Biophysica Acta*.

1989 – 1995 Biomedical Predoctoral Research Fellow, Mayo Medical Center, Rochester, Minnesota. During the course of the project, studied the causative role of toxic tannin dust exposure in developing the chronic pulmonary inflammatory disease, Byssinosis. Signal transduction pathways in phospholipase activation and eicosanoid synthesis and lipid second messenger metabolism in human and animal cell models were examined using a strategy combining recently developed mass spectrometry and fluorescent spectroscopy methods, his work was first to quantify fatty acid recycling by measuring endogenous ^{18}O incorporation into fatty acids during deacylation and reacylation accompanying the inflammatory response. Determination of the absolute mass of fatty acids deacylated vs. the actual mass released from agonist-stimulated cells was a unique and novel achievement.

1986 – 1989 Research Biologist, HNRC, USDA. As the head of the methods development section, Dr. Ralston initiated and conducted numerous independent research collaborations, developing tests for human studies including metalloenzyme assays, trace element analytical methods, and cell isolation/purification techniques. His responsibilities included designing and developing metabolic balance studies, mass spec sample preparation for stable isotope analysis, and studies of copper and zinc physiology in animal and human platelet and leukocyte preparations.

1979 – 1986 Chemist, UND. Dr. Ralston was initially responsible for performing sample preparation, laboratory hematology, and clinical chemistry assays. He eventually became responsible for methods development in support of human nutrition research and finally became the methods development supervisor, testing and developing new vitamin, mineral, and enzyme assays on animals and animal

samples in preparation for human studies as well as applying these assays in prototype human samples. Based on prior experience with laboratory design, he participated in designing the preparation and analytical sections of the new trace element analysis suite at HNRC.

Publications and Presentations

- Has authored or coauthored numerous publications

DR. STEVEN A. BENSON
Senior Research Manager/Advisor
Energy & Environmental Research Center (EERC)
University of North Dakota (UND)
PO Box 9018, Grand Forks, ND 58202-9018 USA
Phone (701) 777-5000 Fax (701) 777-5181
E-Mail: sbenson@undeerc.org

Principal Areas of Expertise

Development and management of complex multidisciplinary programs focused on solving environmental and energy problems, including 1) technologies to improve the performance of combustion/gasification and associated air pollution control systems; 2) transformations and control of air toxic substances in combustion and gasification systems; 3) advanced analytical techniques to measure the chemical and physical transformations of inorganic species in gases; 4) computer-based models to predict the emissions and fate of pollutants from combustion and gasification systems; 5) advanced materials for power systems; 6) impacts of power system emissions on the environment; 7) national and international conferences and training programs; and 8) state and national environmental policy.

Qualifications

Ph.D., Fuel Science, Materials Science and Engineering, The Pennsylvania State University, 1987.
B.S., Chemistry, Moorhead State University (Minnesota), 1977.

Professional Experience

1999 – Senior Research Manager/Advisor, EERC, UND. Dr. Benson is responsible for leading a group of about 30 highly specialized scientists and engineers whose aim is to develop and conduct projects and programs on power plant performance, environmental control systems, the fate of pollutants, computer modeling, and health issues for clients worldwide. Efforts have focused on the development of multiclient jointly sponsored centers or consortia that are funded by a combination of government and industry sources. Current research activities include computer modeling of combustion and environmental control systems, performance of selective catalytic reduction technologies for NO_x control, carbon-based NO_x reduction technologies, mercury control technologies, particulate matter analysis and source apportionment, the fate of mercury in the environment, toxicology of particulate matter, and in vivo studies of mercury–selenium interactions. The computer-based modeling efforts utilize various kinetic, thermodynamic, artificial neural network, statistical, computation fluid dynamics, and atmospheric dispersion models. These models are used in combination with models developed at the EERC to predict the impacts of fuel properties and system operating conditions on system efficiency and emissions. Dr. Benson is Program Area Manager for Modeling and Database Development for the U.S. Environmental Protection Agency (EPA) Center for Air Toxic Metals^{SM} (CATM$^{®}$) at the EERC. He is responsible for identifying research opportunities and preparing proposals and reports for clients.

1994 – 1999 Associate Director for Research, EERC, UND. Dr. Benson was responsible for the direction and management of programs related to integrated energy and environmental systems development. Dr. Benson led a team of over 45 scientists, engineers, and technicians. In addition, faculty members and graduate students from Chemical Engineering, Chemistry, Geology, and Atmospheric Sciences have been involved in conducting research projects. The research, development, and demonstration programs involve fuel quality effects on power system performance, advanced power systems development/demonstration, computational modeling, advanced materials for power systems, and analytical methods for the characterization of materials. Specific areas of focus included the development and direction of EPA CATM® at the EERC (CATM®, a peer-reviewed, EPA-designated Center of Excellence, is currently in its 12th year of operation and has received funding of over $12,000,000 from government and industry sources), ash behavior in combustion and gasification systems, hot-gas cleanup, and analytical methods of analysis. He was responsible for the identification of research opportunities and the preparation of proposals and reports for clients. Dr. Benson left this position to focus efforts on Microbeam Technologies' Small Business Innovation Research (SBIR).

1986 – 1994 Senior Research Manager, Fuels and Materials Science, EERC, UND. Dr. Benson was responsible for management and supervision of research on the behavior of inorganic constituents, including air toxic metals during combustion and gasification, hot-gas cleanup (particulate gas-phase species control), fundamental combustion, and analytical methods of inorganic analysis, including SEM and microprobe analysis, Auger, XPS, SIMS, XRD, and XRF. Responsible for identification of research opportunities, preparation of proposals and reports for clients, and publication.

1989 – 1991 Assistant Professor (part-time), Department of Geology and Geological Engineering, UND. Dr. Benson was responsible for teaching courses on coal geochemistry, coal ash behavior in combustion and gasification systems, and analytical methods of materials analysis. Taught courses on SEM/microprobe analysis and mineral transformations during coal combustion.

1984 – 1986 Graduate Research Assistant, Fuel Science Program, Department of Materials Science and Engineering, The Pennsylvania State University.

1983 – 1984 Research Supervisor, Distribution of Inorganics and Geochemistry, Coal Science Division, UND Energy Research Center. Dr. Benson was responsible for management and supervision of research on the distribution of major, minor, and trace inorganic constituents and geochemistry of coals and ash chemistry related to inorganic constituents and mineral interactions and transformations during coal combustion and environmental control systems.

1980 – 1983 Research Chemist, U.S. Department of Energy (DOE) Grand Forks Energy Technology Center. Dr. Benson performed research on surface and/or chemical

analysis and characterization of coal-derived materials by SEM, XRF, and thermal analysis in support of projects involving SO_x, NO_x, and particulate control; ash deposition; heavy metals in combustion systems; coal gasification; and fluidized-bed combustion.

1979 – 1980 Research Chemist, DOE Grand Forks Energy Technology Center. Dr. Benson performed research on the application of such techniques as differential thermal analysis, differential scanning calorimetry, thermogravimetric analysis, and energy-dispersive XRF analysis with application to low-rank coals and coal process-related material. In addition, research was performed on the use of x-ray analysis to measure trace elements in fuels and conversion products.

1977 – 1979 Chemist, DOE Grand Forks Energy Technology Center. Dr. Benson performed analysis on coal and coal derivatives by techniques such as wavelength-dispersive x-ray analysis, argon plasma spectrometry, atomic absorption spectrometry, thermal analysis, and elemental analysis (CHN).

1976 – 1977 Teaching Assistant, Department of Chemistry, Moorhead State University.

Professional Memberships and Activities

United States Senate Committee on the Environment and Public Works

- One of three technical panelists invited to provide testimony on mercury control for the coal-fired power industry.
- American Chemical Society (ACS)
  - Chair – Fuel Division 2004 – Duties comprise coordinating all aspects of the division, including publications and national conferences.
  - Fuel Division – Participates on the Executive Committee involved in the coordination and direction of division activities, including outreach, programming, finances, and publications.
  - Councilor, Fuel Division – Represents the Fuel Division at the National ACS Council meeting.
  - Chair Elect, Fuel Division – August 2002 – Elected to be Chair of the Fuel Division.
  - Member, Committee on Environmental Improvement (CEI) – The committee provides advice and direction to the ACS governance on policies and programs related to the environment. Since becoming a member of the committee, we have developed policy statements on Global Climate Change, Reformulated Gasoline and MtBE, and Energy Policy. These policy statements are used to assist legislators in developing national environmental policy. Members of CEI also provide testimony on a variety of environmental issues.
- American Society for Mechanical Engineers (ASME)
  - Advisory Member, ASME Committee on Corrosion and Deposition Resulting from Impurities in Gas Streams. Developed several conferences through the International Engineering Foundation.
- Mercury Reduction Initiative – Minnesota Pollution Control Agency (MPCA)

- Participated in meetings for the mercury reduction initiative and provided advice regarding mercury control technologies for electric utilities and MPCA for voluntary mercury reduction strategies.

♦ Elsevier Science, *Fuel Processing Technology*
- Editorial board member whose role is to provide advice and direction for the journal.

Publications and Presentations
- Has authored/coauthored over 210 publications and is the editor of six books and *Fuel Processing Technology* special issues.

DR. LAURA J. RAYMOND
Postdoctoral Research Associate
Energy & Environmental Research Center (EERC)
University of North Dakota (UND)
PO Box 9018, Grand Forks, ND 58202-9018 USA
Phone (701) 777-5066 Fax (701) 777-5181
E-Mail: lraymond@undeerc.org

Principal Areas of Expertise

Dr. Raymond's principal areas of interest and expertise include evaluating the effects of toxic mercury exposures in selenium-dependent physiology; analyzing the effects of environmental toxins and particulates at the biochemical and molecular levels; and the impact of pollutants on health and physiological processes as well as strategies for prevention, protection, and remediation.

Qualifications

Ph.D., Biochemistry and Molecular Biology, University of North Dakota, 2002.
B.S., Microbiology, University of Arizona, 1993.
Technical and Instrumental Experience: cell culture; column chromatography; spectrometry; cytospin analysis; chemical synthesis; western analysis; northern analysis; southern analysis; gel electrophoresis; fluorescence spectrometry; flow cytometry; enzyme assays; cell separation and purification; cellular organelle isolations and purification; animal dissection; phosphoimaging detection; RNA/DNA isolation; minipreps; primer design; PCR; rtPCR; competitive PCR; internal standard synthesis; and transformation of bacteria.

Professional Experience

2002 – Postdoctoral Research Associate, EERC, UND. Dr. Raymond's research examines biochemical and analytical approaches involved in evaluating potential human health effects and risks resulting from environmental exposure to air, water, and food toxins. Current research examines the physiological roles of trace elements in human health, in particular, the role and mechanisms of mercury exposure on selenium physiology. In addition to mercury and selenium, future areas of research will involve the pathophysiological consequences of arsenic-, nickel-, and asthma-related particulate materials.

1996 – 2002 Predoctoral Research Fellow, Biochemistry and Molecular Biology Research, Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Grand Forks, North Dakota. The location and function of the electron transport chain render the mitochondria the primary source for potentially damaging reactive oxygen species in aerobic cells. Normally, the concentration of superoxide anion in mitochondria (MnSOD) is controlled, at least in part, by the sequential action of superoxide dismutase and catalase. When the terminal steps of the electron transport are impaired or inhibited, superoxide anion production may rise to levels that overwhelm the normal protective enzymes. Studies have shown that copper deficiency causes a reduction in the activity of cytochrome-c oxidase, the copper-dependent, terminal respiratory

complex of the electron transport chain, and also causes an increase in the transcriptional rate for MnSOD. It was hypothesized that under conditions of decreased copper, cytochrome-c oxidase function is impaired resulting in an increase in reactive oxygen species (ROS) production and the subsequent increase in MnSOD levels. Dr. Raymond's research was based on testing this hypothesis by analyzing molecular and biochemical properties of a copper-deficient vs. a copper-adequate cell line. In addition to this research, Dr. Raymond discovered an unusual characteristic of this cell line when certain antioxidants were added to the growing media. Under copper-deficient conditions, apoptosis was initiated. This was not seen in copper-adequate cells. These opposing effects of antioxidants have not been reported for the same cell system in current literature. Continued research involved assessing apoptotic characteristics in this cell line grown under the defined conditions. The overall results of the research suggest that copper deficiency reduces cytochrome-c oxidase function which, in turn, causes an increase in ROS production. The ROS affect the cell system through cell-signaling mechanisms and/or through oxidative damage. Presumably, the redox status of the cell is altered, rendering these cells more sensitive to apoptotic stimuli. This is the first time results such as these have been reported and may lead to novel areas of research in free radical signaling, antioxidant mechanisms, and understanding apoptosis.

1994 – 1995 Graduate studies, including research-related courses such as biochemistry, genetics, and nutrition, leading to pursuit of an advanced degree in biochemistry/molecular biology with a major focus on nutritional metabolism.

1981 – 1992 Medic, United States Air Force (USAF) and USAF Reserve. From 1981 to1985, Dr. Raymond was an active-duty Air Force medic and served on an infection control committee. She remained on active reserve status as an Independent Mobilization Augmentee until July of 1990 at which time she was reactivated for 18 months because of the Persian Gulf War. During her entire Air Force service, she was trained and served throughout all areas of a hospital, but the majority of her time was spent as an emergency room medic. Training and experience were extensive, including in the following areas: emergency medicine, clinical, surgical, administration, medical laboratory, oncology, internal medicine, intensive care, pediatrics, optometry, and obstetrics. As a medic, Dr. Raymond's responsibilities were all-inclusive and ranged from minor patient care to specialty tasks such as suturing, casting, minor surgery procedures, emergency cardiology procedures, drug administration, ambulance attendance, and postmortem care. She also gained field experience such as disaster preparedness, triage, and managing MASH units. Formal education was ongoing throughout years of service and included specific procedural education as well as basic knowledge courses, including earning an associates degree in biology through the USAF college.

APPENDIX E

DETAILED BUDGET AND BUDGET NOTES

SUMMARY BUDGET - ALL YEARS

THE CHALLENGES OF MERCURY EXPOSURE AND THE ROLES OF SLENIUM IN THE SOLUTION
NDIC/INDUSTRY/DOE
PROPOSED START DATE: 6/1/04
EERC PROPOSAL #2004-0041

CATEGORY		TOTAL HRS	TOTAL $COST	INDUSTRIAL SHARE HRS	INDUSTRIAL SHARE $COST	NDIC SHARE HRS	NDIC SHARE $COST	EERC JSRP SHARE HRS	EERC JSRP SHARE $COST
TOTAL DIRECT LABOR		1,958	$ 51,149	671	$ 18,373	671	$ 18,373	616	$ 14,403
TOTAL FRINGE BENEFITS	VAR		$ 24,942		$ 9,208		$ 9,208		$ 6,526
TOTAL LABOR			$ 76,091		$ 27,581		$ 27,581		$ 20,929
OTHER DIRECT COSTS									
TRAVEL			$ 5,208		$ 2,604		$ 2,604		$ -
COMMUNICATION - PHONES & POSTAGE			$ 208		$ 92		$ 92		$ 24
OFFICE (PROJECT SPECIFIC SUPPLIES)			$ 560		$ 241		$ 241		$ 78
SUPPLIES			$ 3,231		$ 1,472		$ 1,472		$ 287
GENERAL (FREIGHT, FOOD, MEMBERSHIPS, ETC.)			$ 150		$ 60		$ 60		$ 30
FEES			$ 15,258		$ -		$ -		$ 15,258
TOTAL OTHER DIRECT COST			$ 24,615		$ 4,469		$ 4,469		$ 15,677
TOTAL DIRECT COST			$100,706		$ 32,050		$ 32,050		$ 36,606
FACILITIES & ADMIN. RATE - % OF MTDC		VAR	$ 53,140	56%	$ 17,950	56%	$ 17,950	VAR	$ 17,240
TOTAL ESTIMATED COST			$153,846		$ 50,000		$ 50,000		$ 53,846

NOTE: Due to limitations within the University's accounting system, the system does not provide for accumulating and reporting expenses at the Detailed Budget level. The Summary Budget is presented for the purpose of how we propose, account, and report expenses. The Detailed Budget is presented to assist in the evaluation of the proposal.

DETAILED BUDGET - ALL YEARS

THE CHALLENGES OF MERCURY EXPOSURE AND THE ROLES OF SLENIUM IN THE SOLUTION
NDIC/INDUSTRY/DOE
PROPOSED START DATE: 6/1/04
EERC PROPOSAL #2004-0041

			YEAR ONE		YEAR TWO			INDUSTRIAL		NDIC		EERC JSRP	
		HOURLY	TOTAL		TOTAL		TOTAL	SHARE		SHARE		SHARE	
LABOR	LABOR CATEGORY	RATE	HRS	$COST	HRS	$COST	HRS	$COST	HRS	$COST	HRS	$COST	HRS	$COST
RALSTON, N.	PROJECT MANAGER	$ 32.45	360	$ 11,682	260	$ 8,437	620	$ 20,119	242	$ 7,853	242	$ 7,853	136	$ 4,413
RAYMOND, L.	PRINCIPAL INVESTIGATOR	$ 20.00	350	$ 7,000	260	$ 5,200	610	$ 12,200	242	$ 4,840	242	$ 4,840	126	$ 2,520
BENSON, S.	PRINCIPAL INVESTIGATOR	$ 51.14	96	$ 4,909	40	$ 2,046	136	$ 6,955	60	$ 3,069	60	$ 3,069	16	$ 817
----------	SENIOR MANAGEMENT	$ 50.49	21	$ 1,060	21	$ 1,060	42	$ 2,120	-	$ -	-	$ -	42	$ 2,120
----------	RESEARCH TECHNICIAN	$ 19.15	30	$ 575	30	$ 575	60	$ 1,150	-	$ -	-	$ -	60	$ 1,150
----------	UNDERGRAD-RES.	$ 9.69	200	$ 1,938	200	$ 1,938	400	$ 3,876	100	$ 969	100	$ 969	200	$ 1,938
----------	TECHNICAL SUPPORT SERVICES	$ 14.90	40	$ 596	50	$ 745	90	$ 1,341	27	$ 402	27	$ 402	36	$ 537
			1,097	$ 27,760	861	$ 20,001	1,958	$ 47,761	671	$ 17,133	671	$ 17,133	616	$ 13,495
ESCALATION ABOVE CURRENT BASE			5%	$ 1,388	10%	$ 2,000	VAR	$ 3,388		$ 1,240		$ 1,240		$ 908
TOTAL DIRECT LABOR				$ 29,148		$ 22,001		$ 51,149		$ 18,373		$ 18,373		$ 14,403
FRINGE BENEFITS - % OF DIRECT LABOR - STAFF		53%		$ 14,370		$ 10,531		$ 24,901		$ 9,198		$ 9,198		$ 6,505
FRINGE BENEFITS - % OF DIRECT LABOR - UNDERGRAD-RES.		1%		$ 20		$ 21		$ 41		$ 10		$ 10		$ 21
TOTAL FRINGE BENEFITS				$ 14,390		$ 10,552		$ 24,942		$ 9,208		$ 9,208		$ 6,526
TOTAL LABOR				$ 43,538		$ 32,553		$ 76,091		$ 27,581		$ 27,581		$ 20,929
OTHER DIRECT COSTS														
TRAVEL				$ -		$ 5,208		$ 5,208		$ 2,604		$ 2,604		$ -
COMMUNICATION - PHONES & POSTAGE				$ 136		$ 72		$ 208		$ 92		$ 92		$ 24
OFFICE (PROJECT SPECIFIC SUPPLIES)				$ 280		$ 280		$ 560		$ 241		$ 241		$ 78
SUPPLIES				$ 2,368		$ 863		$ 3,231		$ 1,472		$ 1,472		$ 287
GENERAL (FREIGHT, FOOD, MEMBERSHIPS, ETC.)				$ 50		$ 100		$ 150		$ 60		$ 60		$ 30
ANALYTICAL RESEARCH LAB.				$ 3,465		$ 10,890		$ 14,355		$ -		$ -		$ 14,355
GRAPHICS SUPPORT				$ 441		$ 462		$ 903		$ -		$ -		$ 903
TOTAL OTHER DIRECT COST				$ 6,740		$ 17,875		$ 24,615		$ 4,469		$ 4,469		$ 15,677
TOTAL DIRECT COST				$ 50,278		$ 50,428		$ 100,706		$ 32,050		$ 32,050		$ 36,606
FACILITIES & ADMIN. RATE - % OF MTDC			VAR	$ 26,645	VAR	$ 26,495	VAR	$ 53,140	56%	$ 17,950	56%	$ 17,950	VAR	$ 17,240
TOTAL ESTIMATED COST				$ 76,923		$ 76,923		$ 153,846		$ 50,000		$ 50,000		$ 53,846

DETAILED BUDGET - FEES

THE CHALLENGES OF MERCURY EXPOSURE AND THE ROLES OF SLENIUM IN THE SOLUTION
EERC PROPOSAL #2004-0041

		YEAR ONE		YEAR TWO		ALL YEARS	
ANALYTICAL RESEARCH LAB.	**RATE**	#	**$COST**	#	**$COST**	#	**$COST**
CVAA	$29	30	$ 870	90	$ 2,610	120	$ 3,480
GFAA	$43	30	$ 1,290	90	$ 3,870	120	$ 5,160
MIXED ACID DIGESTION	$38	30	$ 1,140	90	$ 3,420	120	$ 4,560
SUBTOTAL			$ 3,300		$ 9,900		$ 13,200
ESCALATION		5%	$ 165	10%	$ 990	VAR	$ 1,155
TOTAL ANALYTICAL RESEARCH LAB.			$ 3,465		$ 10,890		$ 14,355

GRAPHICS SUPPORT	**RATE**	#	**$COST**	#	**$COST**	#	**$COST**
GRAPHICS (HOURLY)	$42	10	$ 420	10	$ 420	20	$ 840
SUBTOTAL			$ 420		$ 420		$ 840
ESCALATION		5%	$ 21	10%	$ 42	VAR	$ 63
TOTAL GRAPHICS SUPPORT			$ 441		$ 462		$ 903

DETAILED BUDGET - TRAVEL

THE CHALLENGES OF MERCURY EXPOSURE AND THE ROLES OF SLENIUM IN THE SOLUTION
EERC PROPOSAL #2004-0041

RATES USED TO CALCULATE ESTIMATED TRAVEL EXPENSES

DESTINATION	ECON AIRFARE	LODGING	PER DIEM	CAR RENTAL	REGIST.
Washington, DC	$ 800	$ 250	$ 51	$ 60	$ 650

PURPOSE/DESTINATION	NUMBER OF TRIPS	NUMBER OF PEOPLE	NUMBER OF DAYS	AIRFARE	LODGING	PER DIEM	CAR RENTAL	MISC.	REGIST.	TOTAL	
Conference/Washington, DC	1	2	4	$ 1,600	$ 1,500	$ 408	$ 240	$ 160	$ 1,300	$ 5,208	Y2
TOTAL ESTIMATED TRAVEL -YEAR TWO										$ 5,208	

BUDGET NOTES

ENERGY & ENVIRONMENTAL RESEARCH CENTER (EERC)

Background

The EERC is an independently organized multidisciplinary research center within the University of North Dakota (UND). The EERC receives no appropriated funding from the state of North Dakota and is funded through federal and nonfederal grants, contracts, or other agreements. Although the EERC is not affiliated with any one academic department, university academic faculty may participate in a project, depending on the scope of work and expertise required to perform the project.

The proposed work will be done on a cost-reimbursable basis. The distribution of costs between budget categories (labor, travel, supplies, equipment, subcontracts) is for planning purposes only. The principal investigator may, as dictated by the needs of the work, reallocate the budget among approved items or use the funds for other items directly related to the project, subject only to staying within the total dollars authorized for the overall program. The budget prepared for this proposal is based on a specific start date; this start date is indicated at the top of the EERC budget or identified in the body of the proposal. Please be aware that any delay in the start of this project may result in an increase in the budget.

Salaries and Fringe Benefits

As an interdisciplinary, multiprogram, and multiproject research center, the EERC employs an administrative staff to provide required services for various direct and indirect support functions. Direct project salary estimates are based on the scope of work and prior experience on projects of similar scope. Technical and administrative salary charges are based on direct hourly effort on the project. The labor rate used for specifically identified personnel is the current hourly rate for that individual. The labor category rate is the current average rate of a personnel group with a similar job description. For faculty, if the effort occurs during the academic year and crosses departmental lines, the salary will be in addition to the normal base salary. University policy allows faculty who perform work in addition to their academic contract to receive no more than 20% over the base salary. Costs for general support services such as grants and contracts administration, accounting, personnel, and purchasing and receiving, as well as clerical support of these functions, are included in the EERC facilities and administrative cost rate.

Fringe benefits are estimated on the basis of historical data. The fringe benefits actually charged consist of two components. The first component covers average vacation, holiday, and sick leave (VSL) for the EERC. This component is approved by the UND cognizant audit agency and charged as a percentage of direct labor for permanent staff employees eligible for VSL benefits. The second component covers actual expenses for items such as health, life, and unemployment insurance; social security matching; worker's compensation; and UND retirement contributions.

Travel

Travel is estimated on the basis of UND travel policies which can be found at: http://www.und.edu/dept/accounts/employeetravel.html. Estimates include General Services Administration (GSA) daily meal rates. Travel includes scheduled meetings and conference participation as indicated in the scope of work.

Communications (phones and postage)

Monthly telephone services and fax telephone lines are generally included in the facilities and administrative cost. Direct project cost includes line charges at remote locations, long-distance telephone, including fax-related long-distance calls; postage for regular, air, and express mail; and other data or document transportation costs.

Office (project-specific supplies)

General purpose office supplies (pencils, pens, paper clips, staples, Post-it notes, etc.) are provided through a central storeroom at no cost to individual projects. Budgeted project office supplies include items specifically related to the project; this includes duplicating and printing.

Data Processing

Data processing includes items such as site licenses and computer software.

Supplies

Supplies in this category include scientific supply items such as chemicals, gases, glassware, and/or other project items such as nuts, bolts, and piping necessary for pilot plant operations. Other items also included are supplies such as computer disks, computer paper, memory chips, toner cartridges, maps, and other organizational materials required to complete the project.

Instructional/Research

This category includes subscriptions, books, and reference materials necessary to the project.

Fees

Laboratory, analytical, graphics, and shop/operation fees are established and approved at the beginning of the university's fiscal year.

Laboratory and analytical fees are charged on a per sample, hourly, or daily rate, depending on the analytical services performed. Additionally, laboratory analyses may be performed outside the University when necessary.

Graphics fees are based on an established per hour rate for overall graphics production such as report figures, posters for poster sessions, standard word or table slides, simple maps, schematic slides, desktop publishing, photographs, and printing or copying.

Shop and operation fees are for expenses directly associated with the operation of the pilot plant facility. These fees cover such items as training, safety (protective eye glasses, boots, gloves), and physicals for pilot plant and shop personnel.

General

Freight expenditures generally occur for outgoing items and field sample shipments.

Membership fees (if included) are for memberships in technical areas directly related to work on this project. Technical journals and newsletters received as a result of a membership are used throughout

development and execution of the project as well as by the research team directly involved in project activity.

General expenditures for project meetings, workshops, and conferences where the primary purpose is dissemination of technical information may include costs of food (some of which may exceed the institutional limit), transportation, rental of facilities, and other items incidental to such meetings or conferences.

Facilities and Administrative Cost

The facilities and administrative rate (indirect cost rate) included in this proposal is the rate that became effective July 1, 2002. Facilities and administrative cost is calculated on modified total direct costs (MTDC). MTDC is defined as total direct costs less individual items of equipment in excess of $5000 and subcontracts/subgrants in excess of the first $25,000 for each award.